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Maternal cloacal bacteria of striped plateau lizards inhibit fungal growth

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Fungal infection is a common source of egg loss in reptiles, a taxa that rarely expresses egg-tending behaviors or other forms of parental care. In the absence of parental care, one potential mechanism for antifungal egg protection is the vertical transfer of symbiotic bacteria from the maternal cloaca to eggshell surfaces that inhibit fungal growth. We examined the inhibition of four fungal strains isolated from the natural environment of striped plateau lizards (*Sceloporus virgatus*) when challenged against each of seven bacteria strains isolated from female cloacae. Twenty-three of 28 pairwise interactions showed >80% inhibition of fungal growth, with 18 pairs showing >95% inhibition, similar to *Serratia marcescens* D1, a lab strain known to have potent antifungal activity. Whole genome sequencing of one cloacal strain (*Serratia marcescens* strain 1) identified 6 genes with antifungal products that may break down or outcompete fungi, including Chitinase B and ferri- bacillibactin esterase, and further identified 11 genes that may contribute to biosynthetic pathways for antimicrobial secondary metabolites. Overall, we document strong fungal inhibition by maternal *S. virgatus* cloacal microbes, which are known to transfer to eggshells during oviposition. Comparative work is needed to determine whether vertically-transmitted antifungal microbes are a common source of egg-protection among reptiles, as well as other oviparous species, particularly in the absence of apparent parental care.

KEYWORDS

antifungal, egg protection, *Fusarium*, microbes, nest, reptile, *Sceloporus*, *Serratia*

Introduction

Pathogenic fungi in nest environments have been widely associated with egg mortality in a variety of oviparous animals. For example, [Carvalho et al. \(2019\)](#) determined that *Fusarium* and *Penicillium*, along with other fungi, were responsible for killing the embryos of Tegu lizard eggs. *Fusarium* fungi are also responsible for egg mortality in sea turtles and freshwater turtles ([Sarmiento-Ramírez et al., 2010](#); [Sarmiento-Ramírez et al., 2014](#); [Carranco et al., 2022](#)). Similarly, fungal pathogens have been shown to infect eggs from

Iberian rock lizards and tree frogs, increasing egg mortality, inducing early hatching, and decreasing offspring fitness (Villa, 1979; Warkentin et al., 2001; Moreira and Barata, 2005).

Fungal infection of eggs can be limited through behavioral means in species that provide parental care. Two common methods to prevent infection are nest tending, which can limit exposure to harmful microbes while vertically transmitting beneficial antibacterial microbes (Ruiz-Castellano et al., 2016; van Veelen et al., 2017), and egg smearing, in which parents coat eggs with antimicrobial secretions or beneficial microbes (Hosokawa et al., 2006; D'Alba and Shawkey, 2015; Martínez-García et al., 2015). Both of these methods are common in birds and insects, and variations on egg smearing have also been observed in squid and salamanders (Lauer et al., 2007; Kerwin et al., 2019; Suria et al., 2020).

However, many species do not provide care for eggs, meaning that any protection provided by parents must be provisioned at the time of oviposition. In sea turtles (*Eretmochelys imbricata*; Sarmiento-Ramirez et al., 2014) and striped plateau lizards (*Sceloporus virgatus*; Bunker et al., 2021), these protections appear to be sourced from antifungal microbes which reside in the maternal cloaca and are passed to eggs during oviposition – a method that may be far more widespread than currently recognized. The *S. virgatus* cloacal microbiome is largely dominated by gram-negative *Enterobacteriaceae* bacteria, including *Serratia* species (Bunker et al., 2022), which are known to have antifungal properties (Gutiérrez-Román et al., 2015; Dhar Purkayastha et al., 2018). Indeed, eggs that pass through the cloaca, relative to those that are removed by dissection, have reduced fungal attachment and improved hatch success and hatchling phenotype (Bunker et al., 2021).

In this study, we directly tested whether bacterial strains isolated from the cloaca of *S. virgatus* inhibit fungal growth. We used culture-based methods to explore the *in vitro* interactions between several cloacal microbes and potentially pathogenic fungi isolated from the soil of the natural habitat of *S. virgatus*. We aimed to identify both bacterial taxa that may have a protective function in the nest environment and fungal species that may overcome these protections. We measured fungal growth in the presence of cloacal bacteria both qualitatively and quantitatively through spore formation. Additionally, we used whole genome sequencing to identify possible pathways of antifungal action, such as hydrolytic and/or competitive compounds.

Methods

Bacterial isolates

Bacterial isolates were obtained from female *Sceloporus virgatus* cloacal microbes. We captured lizards near the Southwestern Research Station (SWRS) in Cochise County, AZ. This and all work with lizards was approved by the University of Puget Sound IACUC (PS18002) and Arizona Game and Fish (SP649069). Cloacal microbes were sampled using BD ESwab Collection kits; a sterile swab was inserted in the cloaca and gently rotated, then placed in the liquid Amies solution. Microbes were eluted into the

solution, and half the volume was stored at -80°C in a 25% glycerol solution for later use. The remaining volume was used for separate studies (Bunker et al., 2021, 2022). Bacterial samples were grown from frozen stocks on Luria broth (LB) agar plates, and morphologically distinct colonies were restreaked in triplicate to ensure each strain was uniquely isolated. CloneID™ 1X Colony PCR Master Mix (Lucigen) was used to execute whole colony PCR on the 16S rRNA gene from each morphotype isolated, and PCR products were sequenced by Macrogen (Seoul, South Korea). Taxonomy was identified through the National Center for Biotechnology Information (NCBI) BLASTn function (Camacho et al., 2009), and sequences were uploaded to the NCBI database (see Supplementary Table S1 for accession numbers). A sample of each isolated bacterial strain was stored for future use in a 25% glycerol solution at -80°C. These stocks were utilized, as needed, for experimental trials and cultured by incubation in LB media at 30°C while shaking at 300 RPM.

Non-cloacal bacterial strains were also used, to comparatively assess the relative strength of the antifungal properties of cloacal bacteria. These were obtained from previously existing frozen glycerol stocks, and included *Bacillus megaterium*, *Escherichia coli* DH5α, and *Serratia marcescens* D1 (Carolina Biological Supply), the latter of which is known to have antifungal properties and served as a positive control.

Fungal isolates and spore suspensions

Fungal stocks were created by isolating soil fungus that had infected *S. virgatus* eggs. Eggs were obtained from field-collected gravid females induced to oviposit using a 0.1 mL injection of oxytocin. Eggs were laid onto sanitized paper towels, collected with sterile forceps as they were laid, and weighed. Eggs were incubated in a 0.8 mL/g mixture of sterile vermiculite and soil slurry; the slurry was a 1:10 ratio of soil collected near SWRS mixed with sterile water. Eggs that failed to hatch due to fungal fouling were swabbed, fungi on swabs were eluted into Amies solution, and the sample was stored at -80°C in a 25% glycerol solution until plating. Fungal cultures were grown on Malt Extract Agar (MEA) until morphologically distinct mycelia appeared, and unique strains were isolated and cultured in triplicate. Fungi were vouchered as living cultures in sterile water and stored at room temperature. DNA from fungal vouchers was extracted using the Extract-N-Amp™ Tissue PCR Kit (Millipore Sigma). The ITS gene was amplified, then sequenced by Eton Biosciences (San Diego, CA).

Each fungal species (*Fusarium* sp., *Aspergillus* sp., *Neocosmospora rubicola*, and *Purpureocillium lilacinum*) was grown from vouchers on MEA plates infused with three antibiotics: kanamycin (50 µg/mL), ampicillin (100 µg/mL), and tetracycline (15 µg/mL) to discourage bacterial growth. Once fungal lawns covered the agar surface, spore suspensions were created based on Ellwood et al. (2006), as follows. Lawns were flooded with 10 mL of a sterile 5 M NaCl solution, then the plates were incubated at 30°C for approximately 30 min. After incubation, spores were gently agitated using a sterile metal spreader and collected using a

micropipette. Spore suspensions were filtered through sterile cheesecloth to remove hyphal fragments. The concentration of spores in suspensions was determined using a hemocytometer and a dissecting microscope. Spore suspensions were diluted to concentrations of 10^6 spores/mL with the 5 M NaCl solution.

Qualitative assays

Direct bacterial–fungal challenge plates were designed based on Miller et al. (2021). Bacterial strains were incubated in LB media for 48 h at 30°C while shaking at 300 RPM, until cultures reached saturation. Cultures were then diluted to a low optical density (absorbance < 0.10 at 600 nm, determined by Genesys 50 UV-visible spectrometer) with sterile LB. 100 µL of each diluted bacteria culture was mixed with 2 mL of liquid 0.75% LB agar, then poured over a 60 mm x 15 mm solidified LB agar plate. Each plate was inoculated with 10^3 fungal spores using a micropipette. We included a plate with fungus only as a positive control, and a plate without bacteria nor fungus as a negative control. Plates were incubated at 30°C for 4 to 7 days, until the positive control plate showed signs of sporulation (*Fusarium* sp.: 4 d, *Aspergillus* sp.: 6 d, *N. rubicola*: 7 d, *P. lilacinum*: 7 d), and were then photographed and qualitatively assessed for relative fungal growth. Each cloacal bacterial strain was challenged once against each fungal strain, whereas the non-cloacal bacterial strains were only challenged against *P. lilacinum* and *N. rubicola*.

Quantitative assays

Fungal inhibition assays were created using a protocol modified from Miller et al. (2021). Bacteria strains were grown to their maximum optical density, then diluted (absorbance < 0.10 at 600 nm) with sterile LB. Then 10^3 spores of a given fungal strain (*Fusarium* sp., *Aspergillus* sp., *N. rubicola*, and *P. lilacinum*) were combined with 100 µL of each bacterial isolate, creating bacterial–fungal liquid cocultures. A liquid culture containing 100 µL of sterile LB and 10^3 fungal spores, as well as a LB plate inoculated with 10^3 fungal spores were prepared as positive controls, and 100 µL of sterile LB only was prepared as a negative control. Liquid cultures were incubated at 30°C while shaking at 300 RPM. Incubation was considered complete after the fungal control plate demonstrated signs of sporulation. At that time, the number of spores in each liquid culture was counted using a hemocytometer and a dissecting microscope. Cloacal bacterial strains were tested with all four fungal strains, and non-cloacal bacterial strains were challenged against *P. lilacinum* and *N. rubicola*. Assays were replicated 5 times per bacterial strain for *P. lilacinum* and *N. rubicola*, and 3 times per bacterial strain for *Fusarium* sp. and *Aspergillus* sp.

Data analysis

For each fungal strain, we performed one-way ANOVAs to compare the concentration of spores across bacterial treatments; tests were run separately for cloacal bacterial strains and for non-

cloacal lab strains. When incubated with cloacal bacterial strains, spore counts for *Fusarium* and *P. lilacinum* were square root transformed to meet parametric assumptions, and a Dunnett's *post hoc* test (Signorell, 2025) was used to compare the average concentration of spores in each bacterial treatment to the average concentration of spores in the positive control. Spore counts for *P. lilacinum* when incubated with non-cloacal strains were log transformed to meet assumptions and analyzed in the same way. Spore counts for *Aspergillus* and *N. rubicola* did not meet parametric assumptions following standard transformations, and were instead analyzed with Kruskal-Wallis tests followed by pairwise Dunn's tests comparing each bacterial strain to the positive control. P-values were adjusted with a Bonferroni adjustment to account for the number of tests. All analyses were performed using R (version 4.0.2, R Core Team 2020). Data and scripts are available at zenodo.org (doi: 10.5281/zenodo.15556854).

Whole genome sequencing

A sample of cloacal *Serratia marcescens* strain 1, which showed strong inhibition of all four fungi, was sent to the Microbial Genome Sequencing Center (MiGS, Pittsburgh, PA) for whole genome sequencing to identify potential genes that could account for antifungal properties. They utilized Illumina and Oxford Nanopore (ONT) sequencing technology to produce raw reads for processing. MiGS assembled and annotated the reads. Quality control and adapter trimming was performed with bcl2fastq and porechop (Wick, 2018), which linked annotations directly to the associated Uniprot entry (Uniprot Consortium), for Illumina and ONT sequencing, respectively. Hybrid assembly with Illumina and ONT reads was performed with Unicycler (Wick, 2017). Assembly statistics were recorded with QUAST (Gurevich et al., 2013). Assembly annotation was performed with Prokka (Seemann 2014). The genome sequence was deposited at DDBJ/ENA/GenBank under the accession JBNRUO000000000. The version described in this paper is version JBNRUO010000000.

UniProt was utilized to research putative genes identified by the annotation process. 16S rRNA sequences were matched to existing organisms using BLAST. Genes that produce secondary metabolites with antimicrobial capabilities were identified using antiSMASH (Blin et al., 2019). Strict alignment parameters were used to identify putative gene clusters.

Results

Sequencing of the 16s rRNA gene and BLASTn comparison identified the seven bacterial strains isolated from the *S. virgatus* cloacal microbiome as the following: *Citrobacter amalonaticus*, *Citrobacter* sp., *Enterobacter ludwigii*, two strains of *Enterococcus faecalis*, and two strains of *Serratia marcescens* (Supplementary Table S1). Fungal strains identified from ITS sequencing were: *Aspergillus* sp., *Fusarium* sp., *Purpureocillium lilacinum*, and *Neocosmospora rubicola*.

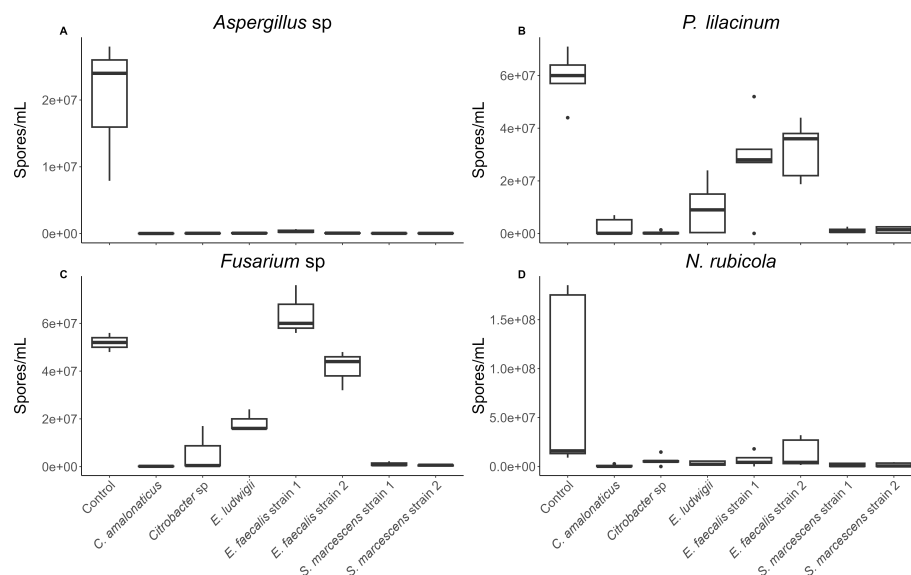


FIGURE 1

Number of (A) *Aspergillus*, (B) *P. lilacinum*, (C) *Fusarium*, and (D) *N. rubicola* spores/mL counted when fungus was incubated individually (control) or with several *Sceloporus virgatus* cloacal bacteria strains. Boxes indicate median and quartiles, and whiskers indicate 95% confidence intervals. $N = 3$ assays/bacterial strain for *Aspergillus* and *Fusarium*. $N = 5$ assays/bacterial strain for *P. lilacinum* and *N. rubicola*. Incubation conditions significantly affected growth of each fungal strain ($p \leq 0.012$). All fungal-bacterial combinations successfully reduced fungal growth except *P. lilacinum* and *Fusarium* competed against *E. faecalis*. P values from post-hoc tests are in Table 1.

Viability of fungi when plated with cloacal bacteria

Aspergillus grew to similar size on the control plate and the plates with the two *E. faecalis* strains, but did not grow on the plates with the other five cloacal bacterial strains (Supplementary Figure S1). *P. lilacinum* and *N. rubicola* grew to similar size on the control plate and the plate with *E. faecalis* strain 1, and did not grow on the plates with the other six cloacal bacterial strains; note that *E. faecalis* strain 2 appeared to swarm when plated with these two fungi (Supplementary Figure S1). *Fusarium*, our fastest growing fungus, grew to similar size on the control plate and the two *E. faecalis* plates, grew a small colony on the *C. amalonaticus* plate, and did not grow when plated with the remaining four cloacal bacterial strains (Supplementary Figure S1).

When interacting with the non-cloacal bacteria, both *P. lilacinum* and *N. rubicola* grew on the control plate, the *B. megaterium* treatment plate, and the *E. coli* DH5 α treatment plate, however these fungi did not grow on the *S. marcescens* D1 treatment plate (Supplementary Figure S2).

Fungal spore formation when interacting with cloacal bacteria

Spore formation of each fungal species was individually compared across 7 cloacal bacterial strains and a control, to determine variation in spore formation when in coculture (or control) conditions. When incubated with cloacal bacterial strains, all four fungal strains were found to vary significantly

across bacterial treatments (*Aspergillus*: $\chi^2 = 18.05$, $df = 7$, $p = 0.012$; *P. lilacinum*: $F = 19.09$, $df = 7,32$, $p < 0.001$; *Fusarium*: $F = 43.33$, $df = 7,16$, $p < 0.001$; *N. rubicola*: $\chi^2 = 18.73$, $df = 7$, $p = 0.009$; Figures 1a–d). Post-hoc tests indicated that fungal growth was significantly inhibited in the presence of all seven bacterial strains for *Aspergillus* and *N. rubicola*, and for six and five out of seven bacterial strains for *P. lilacinum* and *Fusarium*, respectively (excluding one or both *E. faecalis* strains), relative to growth seen in control cultures. Bacterial inhibition of fungal growth was extremely successful with percent reductions in fungal growth over 80%, often over 95% (for 18 of 28 combinations), and up to 99.9% (Table 1).

P. lilacinum and *N. rubicola* were also compared against non-cloacal bacterial strains, in the same manner. There were significant differences between treatment types overall (*P. lilacinum*: $F = 184.8$, $df = 3,16$, $p < 0.001$; *N. rubicola*: $\chi^2 = 16.29$, $df = 3$, $p = 0.001$; Figures 2a, b), and between controls and bacterial treatments based on Dunnett's tests. The scale of these changes were substantially smaller when fungi were treated with *B. megaterium* and *E. coli* DH5 α , but similar to the cloacal bacteria when treated with *S. marcescens* D1, which is known to have antifungal properties (Table 2).

Potential antifungal pathway

To look for gene loci associated with antifungal activity, we selected *Serratia marcescens* strain 1 for whole genome sequencing. Sequencing yielded 30 contigs with an N50 value of 1.55 Mbp (largest contig: 1.89 Mbp) and there were no ambiguous bases. GC

TABLE 1 Percent reduction of fungal spores in bacterial challenges compared to the control, sample sizes, and p-values from *post-hoc* tests comparing each bacterial treatment to the control treatment.

Fungus	<i>Citrobacter amalonaticus</i>	<i>Citrobacter</i> sp	<i>Enterobacter ludwigii</i>	<i>Enterococcus faecalis</i> strain 1	<i>Enterococcus faecalis</i> strain 2	<i>Serratia marcescens</i> strain 1	<i>Serratia marcescens</i> strain 2
<i>Aspergillus</i> sp.	99.9% n = 3 p = 0.007	99.8% n = 3 p = 0.007	99.6% n = 3 p = 0.007	98.2% n = 3 p = 0.007	99.7% n = 3 p = 0.007	99.8% n = 3 p = 0.007	99.8% n = 3 p = 0.007
<i>P. lilacinum</i>	95.8% n = 5 p < 0.001	99.4% n = 5 p < 0.001	83.6% n = 5 p < 0.001	53.0% n = 5 p = 0.010	46.4% n = 5 p = 0.100	98.1% n = 5 p < 0.001	97.6% n = 5 p < 0.001
<i>Fusarium</i> sp.	99.8% n = 3 p < 0.001	88.6% n = 3 p < 0.001	64.1% n = 3 p = 0.003	-23.1% n = 3 p = 0.768	20.5% n = 3 p = 0.743	97.9% n = 3 p < 0.001	99.1% n = 3 p < 0.001
<i>N. rubicola</i>	99.0% n = 5 p = 0.001	92.4% n = 5 p = 0.004	96.0% n = 5 p = 0.001	91.3% n = 5 p = 0.007	83.0% n = 5 p = 0.025	98.1% n = 5 p = 0.001	97.8% n = 5 p = 0.001

Bacteria were isolated from the cloaca of *Sceloporus virgatus*. Shading indicates strength of antifungal action (red: >95% inhibition; orange: 90-95% inhibition; dark yellow: 80-90% inhibition; light yellow: 60-80% inhibition).

content was in the expected range for bacterial genomes (59.3%), total assembly length was 5.9 Mbp, and estimated coverage was around 80x. Analysis of the *Serratia marcescens* strain 1 genome identified 6 genes with potential antifungal products (Table 3), including the gene pathway for Chitinase B and ferri-bacillibactin esterase. Further, we identified 11 genomic regions that bear resemblance to biosynthetic gene clusters that produce antimicrobial secondary metabolites (Supplementary Table S2). Most of the identified secondary metabolite-producing genes have products that have been characterized to have some antimicrobial properties, although not fungus-specific.

Discussion

Both qualitative and quantitative results indicate that cloacal bacteria of *S. virgatus* females can significantly inhibit fungal growth, supporting previous evidence (Bunker et al., 2021) that these bacteria offer antifungal protection to eggs in the nest environment. The two methodological approaches provided some different patterns across the various bacterial-fungal combinations. For example, *Aspergillus* was more successful against the two strains of *E. faecalis* when growing on plates than when growing in liquid culture. In contrast, *P. lilacinum* was less successful against

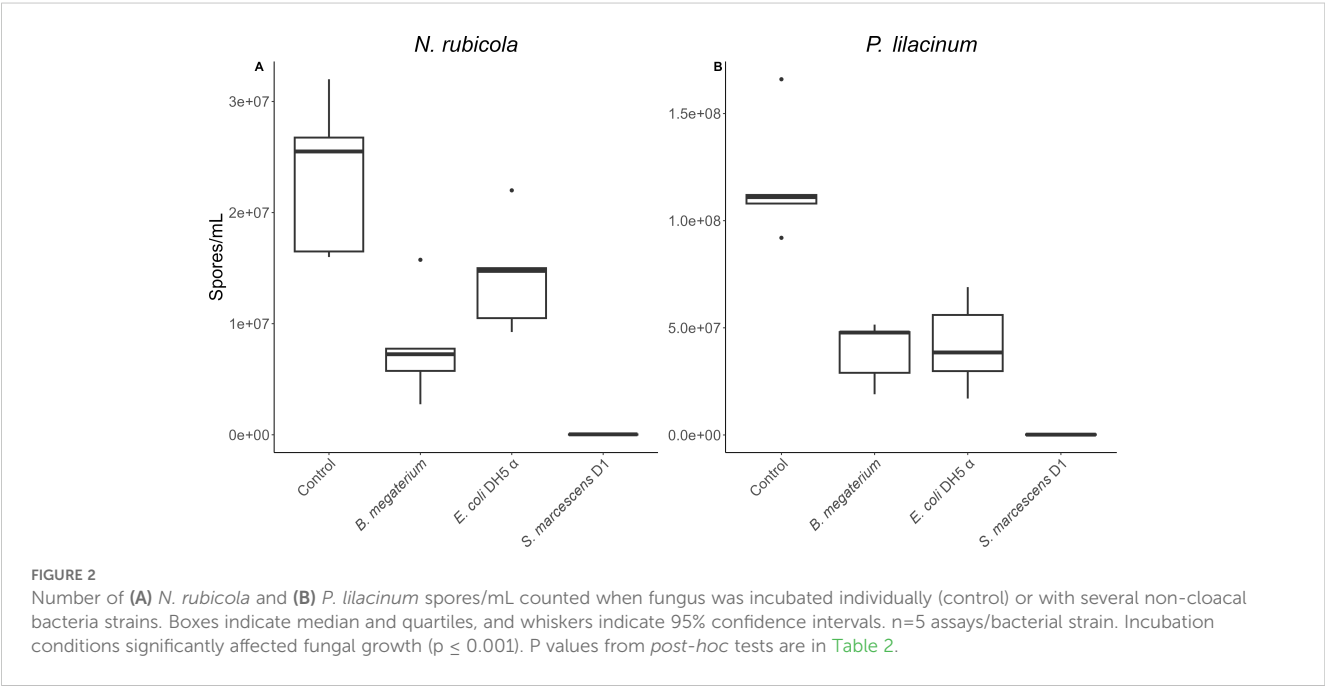


TABLE 2 Percent reduction in fungal spore counts in each non-cloacal bacterial treatment compared to the control, sample sizes, and p-values from *post-hoc* tests comparing each bacterial treatment to the control treatment.

Fungus	<i>B. megaterium</i>	<i>E. coli</i> DH5 α	<i>S. marcescens</i> D1
<i>P. lilacinum</i>	66.8% n = 5 p = 0.005	64.3% n = 5 p = 0.006	99.8% n = 5 p < 0.001
<i>N. rubicola</i>	66.4% n = 5 p = 0.009	38.8% n = 5 p = 0.028	99.9% n = 5 p = 0.009

Shading indicates strength of antifungal action (red: >95% inhibition; light yellow: 60–80% inhibition; tan: 40–60% inhibition).

E. faecalis strain 2 when grown on plates than when grown in liquid culture, perhaps due to the swarming behavior of this strain when grown on surfaces.

Based on inhibition of spore formation, *Aspergillus* was the most sensitive fungi, with fungal spore formation significantly reduced (>95% inhibition) when cultured with each of the seven cloacal bacterial strains. *N. rubicola* was also significantly reduced when challenged with all seven cloacal bacterial strains (>80% inhibition). The most successful bacterial strains were *C. amalonaticus* and the two *Serratia marcescens* strains, as these strains consistently and strongly reduced fungal growth (>95% inhibition) across all four fungal strains. In contrast, the two *E. faecalis* strains only inhibited two of the four fungal strains. Although non-cloacal strains *B. megaterium* and *E. coli* DH5 α (which served as controls) also reduced fungal spore growth, the scale of the reduction was generally much lower than that produced by the cloacal strains and by the positive control non-cloacal strain,

Serratia marcescens D1. This indicates that, while co-culturing itself can reduce fungal growth, possibly through resource competition, there are additional antifungal mechanisms (i.e., stronger competitive abilities and/or metabolite and protein production) at work in the cloacal strains. Antifungal effects in natural nests, where multiple bacterial taxa are acting in combination, may be even stronger than those documented in our two-species interactions.

Antifungal mechanisms were investigated through whole genome sequencing. We identified several genes that code for products with direct antifungal effects, including chitinase B, a 2-nitroimidazole transporter, and validamycin A dioxygenase. Most notably, chitinases are widely known to facilitate antifungal activity in *Serratia* by breaking down chitin in fungal cell walls (Gutiérrez-Román et al., 2015; Dhar Purkayastha et al., 2018). We have preliminary evidence that five of our seven cloacal bacteria strains (all but the two *E. faecalis* strains) can grow on chitin minimum media, suggesting chitinase activity (Supplementary Figure S3). The 2-nitroimidazole transporter could be beneficial both in defense against antimicrobial compounds for *Serratia*, as well as having an antagonistic effect on other microbes (Seki et al., 1970; Ogasawara et al., 2015). Validomycin A is part of a larger validomycin complex that has known antifungal properties (Guirao-Abad and Sánchez-Fresneda, 2013). We also identified the presence of a gene for ferri-bacillibactin esterase, which has been shown to kill fungus by starving them of environmental iron (Yu et al., 2011), suggesting competitive interactions may underlie the inhibition of fungal growth documented herein. Finally, we identified several secondary metabolites that have general antimicrobial effects, or support pathways with potential antifungal effects (Supplementary Table S1). Future work should examine which of these (and other) genes are upregulated when in the presence of fungi. For instance,

TABLE 3 Six genes of interest identified in the genome of *Serratia marcescens* strain 1, isolated from the cloaca of female *Sceloporus virgatus*.

Gene	Product	Location (nt)	Source organism	Posited function
besA	Ferri-bacillibactin esterase	1,475,187–1,476,101	<i>Bacillus subtilis</i>	Cleaves bacillibactin to use bioavailable iron. ¹ Bacillibactin can be used antagonistically as an antimicrobial ²
chiB	Chitinase B	749,791 – 751,290	<i>Serratia marcescens</i>	Antifungal, breaks down chitinous cell walls of fungi
nimT	2-nitroimidazole transporter	1,192,302–1,193,522	<i>Escherichia coli</i>	Immunity to 2-nitroimidazole ³ which could allow <i>Serratia</i> to export 2-nitroimidazole, an antifungal ⁴
rdmC	Aclacinomycin methylesterase	392,813 – 393,718	<i>Streptomyces purpurascens</i>	Part of the biosynthetic pathway that produces anthracycline, a DNA-intercalating antimicrobial
rebG	4'-demethylrebeccamycin synthase	850,489 – 851,820	<i>Lentzea aerocolonigenes</i>	Final enzyme in the synthesis pathway for the antimicrobial rebeccamycin
vldW	Validamycin A dioxygenase	1,474,450–1,475,472	<i>Streptomyces hygroscopicus</i> subsp. <i>limoneus</i>	Creates part of the validamycin complex which has potent antifungal and antimicrobial activity ⁵

Gene product, location in the genome, source organisms, and potential function in *Serratia* are included. Source organism and potential function were extrapolated from UniProt database.

¹Miethke et al., 2006.

²Chakraborty et al., 2022.

³Ogasawara et al., 2015.

⁴Mital, 2009.

⁵Plabutong et al., 2020.

Serratia strains isolated from amphibian skin are known to confer resistance to the global fungal pathogen, *Batrachochytrium dendrobatidis* (*Bd*), implicated in global amphibian declines; when co-cultured with *Bd* these strains show upregulation of genes associated with nitrate reductases, nitrite transporters, and acyl-coenzyme A dehydrogenase supporting their involvement in mediating antifungal action (Madison et al., 2017).

Though the underlying mechanism(s) are unresolved, our findings indicate an effective antifungal function of maternal *S. virgatus* cloacal microbes. The five strains most successful at inhibiting fungal growth (two *Citrobacter*, *Enterobacter*, and two *Serratia*) are all from *Enterobacteriaceae*, the family that dominates the *S. virgatus* cloacal microbiome, comprising 77% of the microbial community during the reproductive season (Bunker et al., 2022). We have previously found that cloacal microbes transfer to eggshells and increase egg survival and hatchling fitness of *S. virgatus* offspring (Bunker et al., 2021; Bunker and Weiss, 2024). A similar phenomenon has been observed in sea turtles, whose eggs are often infected by *Fusarium* (Sarmiento-Ramirez et al., 2014). Maternal transmission of microbiota is widespread across the animal kingdom, including many species that, like the majority of squamate reptiles, are oviparous and lack parental care (Rowe et al., 2020; Nyholm, 2020). Among oviparous species, the microbial nest environment and its maternal contributions are key aspects of the “nidobiome,” which has recently been posited as an integral part of neonate development (Campos-Cerda and Bohannan, 2020).

Despite the apparent efficacy of the *S. virgatus* cloacal microbiome as an antifungal agent, reptile eggs of other species are often killed by fungal infections (Otaño et al., 2014; Badiane et al., 2017; Carvalho et al., 2019; Gleason et al., 2020), and interactions between fungi and cloacal microbes are likely more complex in the natural nest environment than in our *in vitro* cocultures. For instance, in the nest, fungus may experience a more ideal growing environment, and may be able to establish in the nest on unfertilized or failed eggs and then opportunistically transfer to neighboring eggs (Moreira and Barata, 2005). These factors could help to reconcile the very strong inhibitory effects of the maternal microbiome in our experimental system, and known rates of fungal infection and egg failure in natural reptile nests. However, anecdotally, egg failure rates for *S. virgatus* both in natural settings and when challenged with fungus in a lab setting appear to be low (unpublished data). The nesting ecology of this temperate species is relatively unique in that oviposition is triggered by the onset of the North American monsoon rains (Vinegar, 1975). Nesting in periods of increased moisture may have put strong selective pressure on *S. virgatus* for its potent antifungal microbes. Future comparative studies, across reptile taxa, are needed to assess this possibility.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: Zenodo, <https://doi.org/10.5281/zenodo.15556854>.

16S rRNA sequences are uploaded to the NCBI database, with accession numbers listed in [Supplementary Table S1](#). The genome sequence was deposited at DDBJ/ENA/GenBank under the accession JBNRUO000000000.

Ethics statement

The animal study was approved by University of Puget Sound Institutional Animal Care and Use Committee. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

MLB: Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft. OP: Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft. MEB: Data curation, Formal Analysis, Investigation, Visualization, Writing – original draft. MM: Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Supervision, Writing – review & editing. SW: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/famrs.2025.1589793/full#supplementary-material>

SUPPLEMENTARY FIGURE 1

Variation in growth of fungal species when incubated with *Sceloporus virgatus* cloacal bacteria on LB agar. The left column shows fungal growth in the absence of bacteria. Swarming by *E. faecalis* strain 2 was observed when paired with *P. lilacinum* and *N. rubicola*.

SUPPLEMENTARY FIGURE 2

Variation in growth of fungal species when incubated with non-cloacal bacteria on LB agar. The left column shows fungal growth in the absence of bacteria.

SUPPLEMENTARY FIGURE 3

Growth of seven bacteria isolated from the *Sceloporus virgatus* cloaca on chitin minimum media. Presence of bacterial growth on this media indicates bacterial chitinase activity. The unnumbered section of the plate had no bacteria.

SUPPLEMENTARY TABLE 1

Identities and accession numbers of bacterial strains isolated from the *Sceloporus virgatus* cloacal microbiome.

SUPPLEMENTARY TABLE 2

Serratia marcescens strain 1's putative genes and their antimicrobial secondary metabolites. Output is taken from antiSMASH. The genome region, product type, and base pair location (nt) are given for each putative gene. Accompanying information is for the most similar known cluster and what type of molecule it produces, as well as the molecule's identity and gene cluster's similarity to the sample genome. n/a is assigned to regions with structural similarity to antimicrobial-producing clusters but lack exact sequence matches to functionally characterized clusters.

References

- Badiane, A., Pérez i de Lanuza, G., del C. García-Custodio, M., Carazo, P., and Font, E. (2017). Colour patch size and measurement error using reflectance spectrophotometry. *Methods Ecol. Evol.* 8, 1585–1593. doi: 10.1111/mee3.2017.8.issue-11
- Blin, K., Shaw, S., Steinke, K., Villebro, R., Ziemert, N., Lee, S. Y., et al. (2019). antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. *Nucleic Acids Research* 47, W81–W87. doi: 10.1093/nar/gkz310
- Bunker, M. E., Arnold, A. E., and Weiss, S. L. (2022). Wild microbiomes of striped plateau lizards vary with reproductive season, sex, and body size. *Sci. Rep.* 12, 1–12. doi: 10.1038/s41598-022-24518-6
- Bunker, M. E., Elliott, G., Heyer-Gray, H., Martin, M. O., Arnold, A. E., and Weiss, S. L. (2021). Vertically transmitted microbiome protects eggs from fungal infection and egg failure. *Anim. Microb.* 3, 43. doi: 10.1186/s42523-021-00104-5
- Bunker, M. E., and Weiss, S. L. (2024). The reproductive microbiome and maternal transmission of microbiota via eggs in *Sceloporus virgatus*. *FEMS Microbiol. Ecol.* 100, fae011. doi: 10.1093/femsec/fae011
- Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., et al. (2009). BLAST+: architecture and applications. *BMC Bioinf.* 10, 421. doi: 10.1186/1471-2105-10-421
- Campos-Cerda, F., and Bohannan, B. J. M. (2020). The nidobiome: A Framework for understanding microbiome assembly in neonates. *Trends Ecol. Evol.* 35, 573–582. doi: 10.1016/j.tree.2020.03.007
- Carranco, A. S., Gillingham, M. A. F., Wilhelm, K., Torres, M. L., Sommer, S., and Romo, D. (2022). Transcending sea turtles: First report of hatching failure in eggs of an Amazonian freshwater turtle with symptoms of the fungal emerging disease fusariosis. *Transbound. Emerg. Dis.* 69, e3282–e3288. doi: 10.1111/tbed.14596
- Carvalho, A. M., Souza, L. K. H., Ataides, F. S., and Pêres Junior, A. K. (2019). Eggs of tegu lizard, *Salvator merianae* (Duméril & Bilbron 1839) (Squamata; Teiidae) damaged by fungal infections. *Braz. J. Biol.* 80, 112–114. doi: 10.1590/1519-6984.193388
- Chakraborty, K., Kizhakkekalam, V., Joy, M., and Chakraborty, R. (2022). Bacillibactin class of siderophore antibiotics from a marine symbiotic *Bacillus* as promising antibacterial agents. *Appl. Microbiol. Biotechnol.* 106, 329–340. doi: 10.1007/s00253-021-11632-0
- D'Alba, L., and Shawkey, M. D. (2015). Mechanisms of antimicrobial defense in avian eggs. *J. Ornithol.* 156, 399–408. doi: 10.1007/s10336-015-1226-1
- Dhar Purkayastha, G., Mangar, P., Saha, A., and Saha, D. (2018). Evaluation of the biocontrol efficacy of a *Serratia marcescens* strain indigenous to tea rhizosphere for the management of root rot disease in tea. *PLoS One* 13, 0191761. doi: 10.1371/journal.pone.0191761
- Ellwood, S., Kamphuis, L., Pfaff, T., Oliver, R., Samac, D. A., Foster-Hartnett, D., et al. (2006). "Inoculation and growth with foliar pathogenic fungi," in *The Medicago truncatula Handbook* (The Samuel Roberts Noble Foundation, Ardmore, OK).
- Gleason, F. H., Allerstorfer, M., and Lilje, O. (2020). Newly emerging diseases of marine turtles, especially sea turtle egg fusariosis (SEFT), caused by species in the *Fusarium solani* complex (FSSC). *Mycology* 11, 184–194. doi: 10.1080/21501203.2019.1710303
- Guirao-Abad, J. P., and Sánchez-Fresneda, R. (2013). Analysis of validamycin as a potential antifungal compound against *Candida albicans*. *Int. Microbiol.* 16, 217–225. doi: 10.2436/20.1501.01.197
- Gurevich, A., Saveliev, V., Vyahhi, N., and Tesler, G. (2013). QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 29, 1072–1075. doi: 10.1093/bioinformatics/btt086
- Gutiérrez-Román, M. I., Holguín-Meléndez, F., Dunn, M. F., Guillén-Navarro, K., and Huerta-Palacios, G. (2015). Antifungal activity of *Serratia marcescens* CFFSUR-B2 purified chitinolytic enzymes and prodigiosin against *Mycosphaerella fijiensis*, causal agent of black Sigatoka in banana (*Musa* spp.). *BioControl* 60, 565–572. doi: 10.1007/s10526-015-9655-6
- Hosokawa, T., Kikuchi, Y., Nikoh, N., Shimada, M., and Fukatsu, T. (2006). Strict host-symbiont cospeciation and reductive genome evolution in insect gut bacteria. *PLoS Biol.* 4, e337. doi: 10.1371/journal.pbio.0040337
- Kerwin, A. H., Gromek, S. M., Suria, A. M., Samples, R. M., Deoss, D. J., O'Donnell, K., et al. (2019). Shielding the next generation: Symbiotic bacteria from a reproductive organ protect bobtail squid eggs from fungal fouling. *mBio* 10, 10.1128/mbio.02376-19. doi: 10.1128/mbio.02376-19
- Lauer, A., Simon, M. A., Banning, J. L., André, E., Duncan, K., and Harris, R. N. (2007). Common cutaneous bacteria from the Eastern red-backed salamander can inhibit pathogenic fungi. *Copeia* 2007, 630–640. doi: 10.1643/0045-8511(2007)2007[630:CCBFTE]2.0.CO;2
- Madison, J. D., Berg, E. A., Abarca, J. G., Whitfield, S. M., Gorbatenko, O., Pinto, A., et al. (2017). Characterization of *Batrachochytrium dendrobatidis* inhibiting bacteria from amphibian populations in Costa Rica. *Front. Microbiol.* 8. doi: 10.3389/fmicb.2017.00290
- Martínez-García, Á., Soler, J. J., Rodríguez-Ruano, S. M., Martínez-Bueno, M., Martín-Platero, A. M., Juárez-García, N., et al. (2015). Preening as a vehicle for key bacteria in hoopes. *Microbiol. Ecol.* 70, 1024–1033. doi: 10.1007/s00248-015-0636-1
- Miethke, M., Klotz, O., Linne, U., May, J. J., Beckering, C. L., and Marahiel, M. A. (2006). Ferri-bacillibactin uptake and hydrolysis in *Bacillus subtilis*. *Mol. Microbiol.* 61, 1413–1427. doi: 10.1111/j.1365-2958.2006.05321.x
- Miller, D. L., Smith, E. A., and Newton, I. L. G. (2021). A bacterial symbiont protects honey bees from fungal disease. *mBio* 12, 10.1128/mbio.00503-21. doi: 10.1128/mbio.00503-21
- Mital, A. (2009). Synthetic nitroimidazoles: biological activities and mutagenicity relationships. *Sci. Pharm.* 77, 497–520. doi: 10.3797/sciparm.0907-14

- Moreira, P. L., and Barata, M. (2005). Egg mortality and early embryo hatching caused by fungal infection of Iberian rock lizard (*Lacerta monticola*) clutches. *Herpetol. J.* 15, 265–272.
- Nyholm, S. V. (2020). In the beginning: egg–microbe interactions and consequences for animal hosts. *Philos. Trans. R. Soc. B: Biol. Sci.* 375, 20190593. doi: 10.1098/rstb.2019.0593
- Ogasawara, H., Ohe, S., and Ishihama, A. (2015). Role of transcription factor NimR (YeaM) in sensitivity control of *Escherichia coli* to 2-nitroimidazole. *FEMS Microbiol. Lett.* 362, 1–8. doi: 10.1093/femsle/fnu013
- Otaño, N. B. N., Piña, C. I., Bucinsky, A. M., and Arambarri, A. M. (2014). Fungal diversity on broad-snouted caiman (*Caiman latirostris*) eggs, and their effects on hatchlings. *Herpetol. J.* 42, 217–222.
- Plabutong, N., Ekronarongchai, S., Niwetbowornchai, N., Edwards, S. W., Virakul, S., Chiewchengchol, D., et al. (2020). The inhibitory effect of validamycin A on *Aspergillus flavus*. *Int. J. Microbiol.* 2020, 397415. doi: 10.1155/2020/3972415
- R Core Team. (2020). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. <https://www.R-project.org/jo>.
- Rowe, M., Veerus, L., Trosvik, P., Buckling, A., and Pizzari, T. (2020). The Reproductive Microbiome: An emerging driver of sexual selection, sexual conflict, mating systems, and reproductive isolation. *Trends Ecol. Evol.* 35, 220–234. doi: 10.1016/j.tree.2019.11.004
- Ruiz-Castellano, C., Tomás, G., Ruiz-Rodríguez, M., Martín-Gálvez, D., and Soler, J. J. (2016). Nest material shapes eggs bacterial environment. *PLoS One* 11, e0148894. doi: 10.1371/journal.pone.0148894
- Sarmiento-Ramírez, J. M., Abella, E., Martín, M. P., Tellería, M. T., López-Jurado, L. F., Marco, A., et al. (2010). *Fusarium solani* is responsible for mass mortalities in nests of loggerhead sea turtle, *Caretta caretta*, in Boavista, Cape Verde. *FEMS Microbiol. Lett.* 312, 192–200. doi: 10.1111/j.1574-6968.2010.02116.x
- Sarmiento-Ramírez, J., van der Voort, M., Raaijmakers, J., and Dieguez-Uribeondo, J. (2014). Unraveling the microbiome of eggs of the endangered sea turtle *Eretmochelys imbricata* identifies bacteria with activity against the emerging pathogen *Fusarium falciforme*. *PLoS One* 9, e95206. doi: 10.1371/journal.pone.0095206
- Seemann, T. (2014). Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30, 2068–2069. doi: 10.1093/bioinformatics/btu153
- Seki, Y., Nakamura, T., and Okami, Y. (1970). Accumulation of 2-aminoimidazole by *Streptomyces eurocidicus*. *J. Biochem.* 67, 389–396. doi: 10.1093/oxfordjournals.jbchem.a129262
- Signorell, A. (2023). DescTools: tools for descriptive statistics. *R package version 0.99.59*, <https://github.com/AndriSignorell/DescTools/>, <https://andrisignorell.github.io/DescTools/>.
- Suria, A. M., Tan, K. C., Kerwin, A. H., Gitzel, L., Abini-Agbomson, L., Bertenshaw, J. M., et al. (2020). Hawaiian bobtail squid symbionts inhibit marine bacteria via production of specialized metabolites, including new bromoalterochromides BAC-D/D'. *mSphere* 5, e00166–e00120. doi: 10.1128/msphere.00166-20
- van Veelen, H. P. J., Falcao Salles, J., and Tieleman, B. I. (2017). Multi-level comparisons of cloacal, skin, feather and nest-associated microbiota suggest considerable influence of horizontal acquisition on the microbiota assembly of sympatric woodlarks and skylarks. *Microbiome* 5, 156. doi: 10.1186/s40168-017-0371-6
- Villa, J. (1979). Two fungi lethal to frog eggs in Central America. *Copeia* 1979, 650–655. doi: 10.2307/1443873
- Vinegar, M. B. (1975). Demography of the striped plateau lizard, *Sceloporus virgatus*. *Ecology* 56, 172–182. doi: 10.2307/1935309
- Warkentin, K. M., Currie, C. R., and Rehner, S. A. (2001). Egg-killing fungus induces early hatching of red-eyed treefrog eggs. *Ecology* 82, 2860–2869. doi: 10.1890/0012-9658(2001)082[2860:EKFIEH]2.0.CO;2
- Wick, R. R. (2018). Porechop: Adapter trimmer for Oxford Nanopore reads. [Software]. <https://github.com/rrwick/Porechop>.
- Wick, R. R., Judd, L. M., Gorrie, C. L., and Holt, K. E. (2017). Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Computational Biology* 13, e1005595. doi: 10.1371/journal.pcbi.1005595
- Yu, X., Ai, C., Xin, L., and Zhou, G. (2011). The siderophore-producing bacterium, *Bacillus subtilis* CAS15, has a biocontrol effect on *Fusarium* wilt and promotes the growth of pepper. *Eur. J. Soil Biol.* 47, 138–145. doi: 10.1016/j.ejsobi.2010.11.001